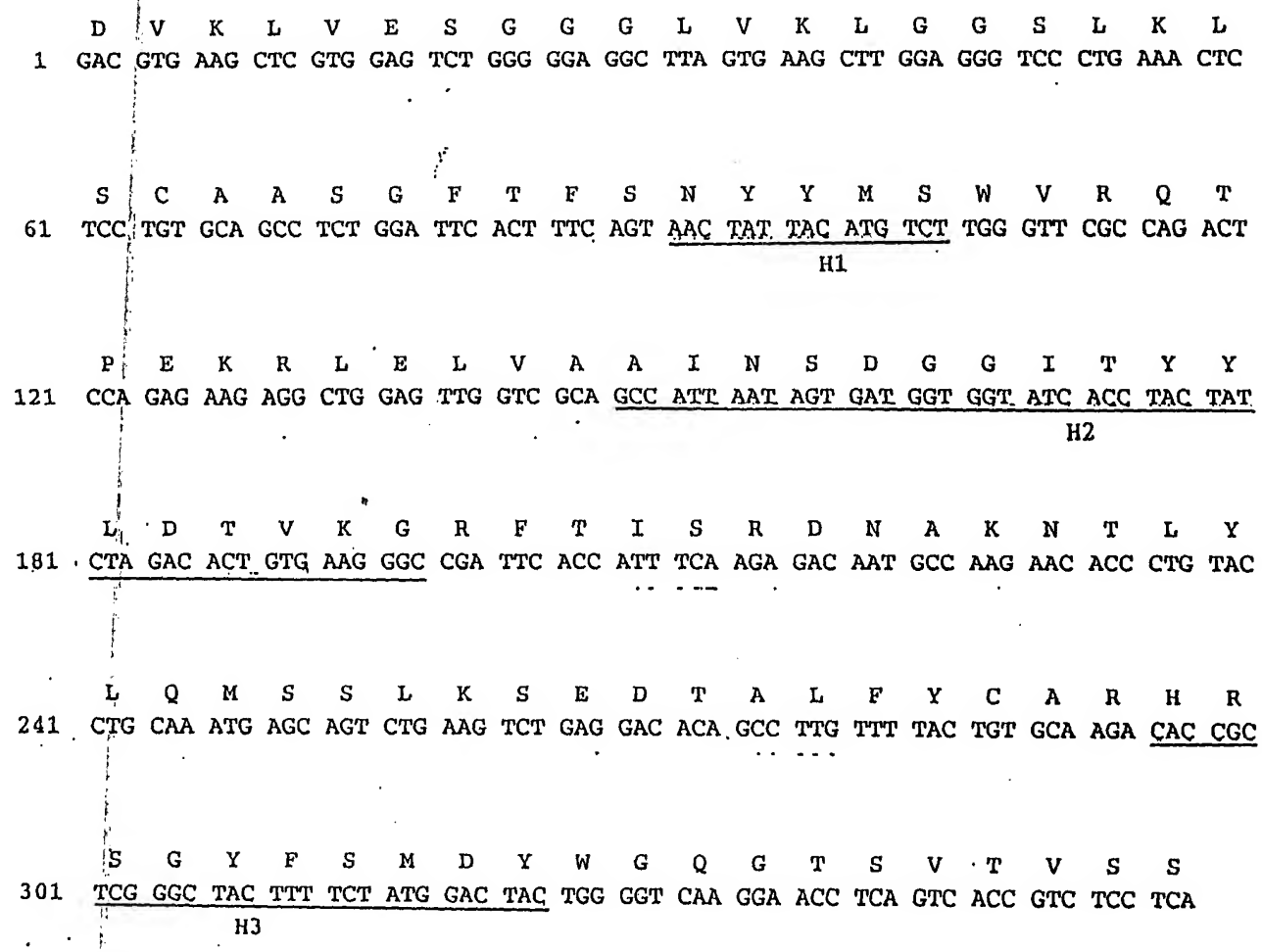
VH G250



CDRs H1, H2, H3

CDR definition according to Kabat scheme

Figure 1

Figure 2

Primers used for PCR amplification of G250 VH and VL regions

Anchor and anchor poly C primers:

Anchor: 5'-GCA TGC GCG CGG CCG CGG AGG CC-3'

Anchor poly C: 5'-GCA TGC GCG CGG CCG CGG AGG $CC(C)_{12}$-3'

Constant primers:

VH-primers: 5'-CTC TAA GCT TGG CTC AAA CAC AGC
GAC CTC GGA TAC AGT TGG TGC AGC-3'

VL-primers: 5'-CTC TTC TAG AGA GTC TCT CAG CTG
GTA GGA TAC AGT TGG TGC AGC-3'

HC_cG250 (EcoRI-fragment in expression vector) double strand sequencing 08.05.2001

Figure 3A

```
   1 TCATGACATT AACCTATAAA AATAGGCGTA TCACGAGGCC CTTTCGTCTT  CAAGAATTCT TCAGATACAA AGAATCTCTA AACCCTGAGG ACATTCTATC 100
 101 ACAAATAAGT AAAATTCAGA AAATTCTGAA TGCTCCCATC ACAGAGATGA  ATCTGCTATG AACAGCTCAT AGGTGTGAAG CTCTACAAAA GCCATATTAT 200
 201 TGAAAAGCCA CATTGTGCCC AGACTTTGGA AAGACTGAGC TCATATCCTG  AAATACAGTT ATGTGTGGTT CTATCTAATT ACACATTTAC ACTAAGGAAA 300
 301 CATGGCAGTA TGGGAATGAA GCTTGTTCTG TACACATTAA CAGAGGGAAA  CTAAACAAAG TATGGTGAAT CCCTAACCAA AAGTAAAAAA AAAAAAAAAA 400
 401 AAGAAAAGAA AAGAAAAAAA AAGTGAAACT ACAATATGTT TCAAATGCTG  TAACTGAAAT CTGGTTTTTT GATGCCTTAT ATCTGTTATC ATCAGTGACT 500
 501 TCAGATTTAG TCCAACTCCA GAGCATGGTA TAGCAGGAAG ACATGCAAAT  AGGTCTTCTC TGTGCCCATG AAAAACACCT CGGCCCTGAC CCTGCAGCTC 600
 601 TGACAGAGGA GGCCTGTCCT GGATTCGATT CCCAGTTCCT CACATTCAGT  GATCAGCACT GAACACAGAC CCCTCACCAT GAACTTCGGG CTCAGATTGA 700
 701 TTTTCCTTGT CCTGGTTTTA AAAGGTATCT TATTGAGTAT AGAGGACATC  TGCTGTATGC ACAGAGGTGC AGAAAAAATG TTGTTTGTTT TTTTTAGTGA 800
 801 CAATGCTCCA AACAGTATTC TTTCTTTGCA GGTGTCCTGT GTGACGTGAA  GCTCGTGGAG TCTGGGGGAG GCTTAGTGAA GCTTGGAGGG TCCCTGAAAC 900
 901 TCTCCTGTGC AGCCTCTGGA TTCACTTTCA GTAACTATTA CATGTCTTGG  GTTCGCCAGA CTCCAGAGAA GAGGCTGGAG TTGGTCGCAG CCATTAATAG 1000
1001 TGATGGTGGT ATCACCTACT ATCTAGACAC TGTGAAGGGC CGATTCACCA  TTTCAAGAGA CAATGCCAAG AACACCCTGT ACCTGCAAAT GAGCAGTCTG 1100
1101 AAGTCTGAGG ACACAGCCTT GTTTTACTGT GCAAGACACC GCTCGGGCTA  CTTTTCTATG GACTACTGGG GTCAAGGAAC CTCAGTCACC GTCTCCTCAG 1200
1201 GTAAGAATGG CCTCTCCAGG TCTTTTTTTT AATCTTTGTA ATGGAGTTTT  CTGAACATTG CAGACTAATC TTGGATATTT GTCCCTGAGG TAGCCGGCTG 1300
1301 AGAGAAATTG GGAATTAAAC TGTCTCGGGA TCTCAGAGCC TTTAGGACAG  ATTATCTCCA CATCTTTGAA AAACTGAGAT TCTGTGTGAT GGTGTTGGTG 1400
1401 GAGTCCCTGG ATGATGGGAT AGGGACTTTG GAGGCTCATT TGAGGGAGAT  GCTAAAACAA TCCTATGGCT GGAGGGAGAG TTGGGGCTGT AGTTGGAGAT 1500
1501 TTTCAGTTTT TAGAATAAAA GCTTTAGCTG CGGGAAATCC TTCAGGACCA  CCTCTGTGAC AGCATTTATA CAGTATCCGA TGCATAGGGA CAAAGAGTGG 1600
1601 AGTGGGGCAC TTTCTTTCGA TTTGTGGGGA ATGTTCCACA CTAGTTTCTG  TGAAACCTCA TTTGTTGGAG GGAGAGCTGT CTTAGTGCCT GAGTCAAGGG 1700
1701 AGAAGGGCAT CTAGCCTCGG TCTCAAAAGG GTAGTTGCTG TCCAGAGAGG  TCTGGTGGAG CCTGCAAAAG TCCAGCTTTC AAAGGAACAC AGAAGTATGT 1800
1801 GTATGGAATA ATAGAAGATG TTGCTTTTAC TCTTAAGTTG GTTCATAGGA  AAAATAGTTA AAACTGTGAG TTTAAAATGT GAGAGGGTTT TCAAGTACTC 1900
1901 ATTTTTTTAC ATGTCCAAAA TTTCTGTCAA TCAATTTGAG GTCTTGTTTG  TGTAGAACTG ACATTACTTA AAGTTTAACC GAGGAATGGG AGTGAGGCTC 2000
2001 TCTCATACCC TATTCAGAAC TGACTTTTAA CAATAATAAA TTAAGTTTAA  AATATTTTTA AATGAATTGA GCAATGTTGA GTTGGAGTCA AGATGGCCGA 2100
```

LC_cG250 (HindIII-fragment in expression vector) double strand sequencing 10.05.2001

```
4501 GAGATCTGGG TCTGACTGCA GGTAGCGTGG TCTTCTAGAC GTTTAAGTGG  GAGATTTGGG GGGGATGAGG AATGAAGGAA CTTCAGGATA GAAAAGGTCT 4600
4601 GAAGTCAAGT TCAGCTCCTA AAATGGATGT GGGAGCAAAC TTTGAAGATA  AACTGAATGA CCCAGAGGAT GAAACAGTGC AGATCAAAGA GGGGCCTGGA 4700
4701 GCTCTGAGAA CAGAAGGAGA GTCATTCGTG TTGAGTTTCC ACAAATACTG  TCTTGAGTTT TGCAATAAAA GTGGGATAGC AGAGTTGAGT GAGCCATAGG 4800
4801 CTGAGTTCTC TCTTTTGTCT CCTAAGTTTT TATGACTACA AAAATCAGTA  GTATGTCCTG AAATAATCAT TAAACTGTTT GAAAGTATGA CTGCTTGCCA 4900
4901 TGTAGATACC ATGGCTTGCT GAATAATCAG AAGAGGTGTG ACTCTTATTC  TAAAATTTGT CACAAAATGT CAAAATGAGA GACTCTGTAG GAACGAGTCC 5000
5001 TTGACAGACA GCTCAAGGGG TTTTTTTCCT TTGTCTCATT TCTACATGAA  AGTAAATTTG AAATGATCTT TTTTATTATA ATAGTAGAAA TACAGTTGGG 5100
5101 TTTGAACTAT ATGTTTTAAT GGCCACGGTT TTGTAAGACA TTTGGCCCTT  TGTTTTCCCA GTTATTACTC GCTTGTAATT TTATATCGCC AGCAATGGAC 5200
5201 TGAAACGGTC CGCAACCTCT TCTTTACAAC TGGGTGACCT CGCGGCTGTG  CCAGCCATTT GGCGTTCACC TTGCCGCTAA GGGCCGTGTG AACCCCCGAG 5300
5301 GTAGCATCCC TTGCTCCGCG TGGACCACTT TCCTGAGGCA CAGTGATAGG  AACAGAGCCA CTAATCTGAA GAGAACAGAG ATGTGACAGA CTACACTAAT 5400
5401 GTTAGAAAAA CAAGGAAAGG GTGACTTATT GGAGATTTCA GAAATAAAAT  GCATTTATTA TTATATTCCC TTATTTTAAT TTTCTATTAG GGAATTAGAA 5500
5501 AGGGCATAAA CTGCTTTATC CAGTGTTATA TTAAAAGCTT TTTTTTTTTC  AGTGCTA                                                5557
```

LC_cG250 (HindIII-fragment in expression vector) double strand sequencing 10.05.2001

Figure 3B

```
   1 AATTCCAAGC TTTGTATCTT CAGATCCAGG AAAGCCACCA CCAATATCAA  ACAGATACAT GCTGAAACCA ACTTCTGTTC TTATGTCAAA TGCACAGCGG 100
 101 GCATCTGACA CTGCCTGCAT GAAGGTCTCA GGTCAATACT TCCACTACAC  ACATGGAAGC TGACACCAAT GACGTCAATA TTTAGCTCTT TTGCCCATTT 200
 201 CAGGAGGAGA CTGCTGGTTT TGAGTGTGGC ACCAGACTTA ACACCAAGTC  GACAAACTGC TTTGGAATCA TCTGTGACAA TCCACAAAAA CAACTTTGTC 300
 301 TTACAATGTG CTCTGACGAC ATTCATCAAT TCATTTCACT GTCAAAAGTC  ATCATCTGGA CTCCATTACT GGCAGCATAC TTGATTTGAG ACACTTGTTT 400
 401 ACAAAAATGT GCATAGGTAA TCCTCTCTGG AGGAACCAGA AGCCCCCGTT  CCAACTGTAT TTCAGTCTTG CTTGCACAGT CAAATCCTGT ACCAATAGCA 500
 501 GCTAGGGTGT TAACTATGGC TCTGTTGTCC TTACACTTGA CTGCACAAAA  AGGAATAACA TTCGGAAGAG CTTTTAGCCA CCTCAGATGC TTCTTTAGAA 600
 601 TGTCTCTGAG GTCCGGAACC TAGAAAGAAG AGACTTCATT TATTATTTTG  TGTTCAGAAT GTCCTTAGCA CTAAAGCCAC CATCTATGAT ACAGCAGTCA 700
 701 AACTCTTCCT TAGTATAGCT GCTCATCGTT CTCCATGTGC CTACAGAAAA  CCTAGACATG GAATTAAATT ATTGCCAGCC CCTTACAAGG TCAACTTATC 800
 801 CAAGAACTGT GAATGCAGAC TCCTTGAAAT GTTGGAAACA CTCACAGCAC  AGGGTCAAGA CTGGCTGGAC ACATGGAGAC ACTGAATCCT GAAGAGCACT 900
 901 TAGCTGTCTG TTGCTTCATC ATGTCTACTG ACCTGAGGTG GCACCAAGCT  GCTTACTGAG GGAGGACTGT GGCGGTGTCT GCAGGAACTG ACAATTCTCC 1000
1001 ACAATTCTCT TACTGCCCCA CTCATAACTC TTCTCTTCTC CATCTTCTTC  TTTCTTTCCT CTCCCCTCCT TTTTCCCTTT CACTACTTTT TTCCTTTCTT 1100
1101 CTTTTCCACT TCCCTTTTCT TTCTTCTTTT GCTGTTGCTG TTGTAAAGGA  TTTATTGTTT CCTCGTGATT GAACCAAAGG TAGTTGTACT ATTATTTCTG 1200
1201 TAAAACTCAT CTGTTGATTT TCTATTAATT AATTAATTTT GTTTACACTC  CATATTTTAT TCAACCCCTC CATCCTCCTA CTGGTCTACA TACCATACCT 1300
1301 CCTTCCCACA CCCCTGTCTC CACATGGATG CTGCCACCTC CCATGCCACC  TGACCTCTCA TCTCCCTAGG GCATCTAGTC TCTTGAGGCT TAGATGCATC 1400
1401 ATTTCTGAGT GAACACAGAT CCAACAATCC TCTGCTATAT GTGTGTTGGT  GGCCTCATAG CAGCTGGTGT ATGCTGCCTG TTTGTTGATC CAGTGTTTGA 1500
1501 GAGGTCTCGC GGGTTCAGAT TAATTGAGAT TGTTGGACCT CCTCAGCGTC  TTTCAGTCTT TCCCTGATTC AACAACAGGG TTCATTGTTT CTGTTCATTG 1600
1601 GTTGGGTGCA AATATCTGCA TCTGACTCAG CTGCTTATTG GGTCTTCTGG  AGTGCAGTCA TGCTAGGTCC GTTTCTATGA GTGCTCCATA GCCTCAGTGA 1700
1701 TAGTGTCAGG CGTTGGGACT GCCCCTTGAC CTGGATTCTA TTTTGGACCT  GTCGCTGGAC CTTCTTTTCC TCAGGCTCCC CTCCATCTGT ATCCCTGTAA 1800
1801 TTCTTTCAGA CAGGAACAAA TATGGGTCAG AGTTGTGAGT GTGGAATGGC  ACCCCCTTCC CTCATTTAAT GCCCTGTCTT CCTGGTGGAA GTGGGCTCTA 1900
1901 TAAGTTCCCA CTCCCTACTG TTGGGCATTT CATCCCTTTG AGTCCTGAGA  GTCTCTCACC TCCCAGGTCT CTGGTGCATT CTGGAGGGTC CTCCCAACCT 2000
2001 CCTACCTCCC CAGGTTGCCT GTTGACAGAC TTCTGCTGGC CCCCAGTGCT  TCAGTCCTTT TCCCTCACCC AATATCTGAT TTGGATGGAA GCCTGTCATG 2100
```

HC_cG250 (EcoRI-fragment in expression vector) double strand sequencing 08.05.2001

```
2101 TCAGAACCAG AACACCTGCA GCAGCTGGCA GGAAGCAGGT CATGTGGCAA  GGCTATTTGG GGAAGGGAAA ATAAAACCAC TAGGTAAACT TGTAGCTGTG 2200
2201 GTTTGAAGAA GTGGTTTTGA AACACTCTGT CCAGCCCCAC CAAACCGAAA  GTCCAGGCTG AGCAAAACAC CACCTGGGTA ATTTGCATTT CTAAAATAAG 2300
2301 TTGAGGATTC AGCCGAAACT GGAGAGGTCC TCTTTTAACT TATTGAGTTC  AACCTTTTAA TTTTAGCTTG AGTAGTTCTA GTTTCCCCAA ACTTAAGTTT 2400
2401 ATCGACTTCT AAAATGTATT TAGAATTCAT T                                                                              2431
```

LC_cG250 (HindIII-fragment in expression vector) double strand sequencing 10.05.2001

```
2101 AGAACATCTA TATACTTGTG GTTTCAGAGC TTTAAATTGG TCCTTGAGCT  TCTATTTTGA GTTCCTTTCC AGTGATTACT TGCTGTCTTT GGTAGTACTT 2200
2201 TTGACTGTTT ATTTAACCTG GATACTCTCA TACAGCTGTG TAATTTACTT  CCTTATTTGA TGACTGCTTT GCATAGATCC CTAGAGGCCA GCCCAGCTGC 2300
2301 CCATGATTTA TAAACCAGGT CTTTGCAGTG AGATCTGAAA TACATCAGAA  CAGCATGGGC TTCAAGATGG AGTTTCATAC TCAGGTCTTT GTATTCGTGT 2400
2401 TTCTCTGGTT GTCTGGTGAG AATTTTAAAA GTATTATAAC ATCTCAAAAG  TAATTTATTT AAATAGCTTT TCCTATAGGA AGCCAATATT AGGCAGACAA 2500
2501 TGCCATTAGA TAAGACATTT TGGATTCTAA CATTTGTGTC AAAAATCTTT  GTATATATAA GTGTTTACTC ATTATCTATT TCTGATTGCA GGTGTTGATG 2600
2601 GAGACATTGT GATGACCCAG TCTCAAAGAT TCATGTCCAC AACAGTAGGA  GACAGGGTCA GCATCACCTG CAAGGCCAGT CAGAATGTGG TTTCTGCTGT 2700
2701 TGCCTGGTAT CAACAGAAAC CAGGACAATC TCCTAAACTA CTGATTTACT  CAGCATCCAA TCGGTACACT GGAGTCCCTG ATCGCTTCAC AGGCAGTGGA 2800
2801 TCTGGGACAG ATTTCACTCT CACCATTAGC AATATGCAGT CTGAAGACCT  GGCTGATTTT TTCTGTCAAC AATATAGCAA CTATCCGTGG ACGTTCGGTG 2900
2901 GAGGCACCAA GCTGGAAATC AAACGTAAAT AGAATCCAAA CTCTCTTTCT  TCCGTTGTCT ATGTCTGTGG CTTCTATGTC TAAAAATGAT GTAGATATTT 3000
3001 TTTCTCTGAG ACCAGATTCT GTCACTCTCC AAGGCAAAGA TACATAGTCA  CTCCGTAAGC AGAGCTGGGA ATAGGCTAGA CATGTTCTCT GGAGAATGAA 3100
3101 TGCCAGTGTA ATAATTAACA CAAGTGATAG TTTCAGAAAT GCTCAAAGAA  GCAGGGTAGC CTGCCCTAGA CAAACCTTTA CTTGGTGCTC AGACCATGCT 3200
3201 CAGTTTTTGT ATGGGGGTTG AGTGAAGGGA CACCAGTGTG TGTATACGTT  CGGAGGGGGG ACCAAGCTGG AAATAAAACG TAAGTTGTCT TCTCAACTCT 3300
3301 TGTTCACTGA GTCTAACCTT GTTACTTTGT TCTTTGTTGT GTGTTTTTCT  TAAGGAGATT TCAGGGATGT ATCAAATTCC ATTCTCAGAT CAGGTGTTAA 3400
3401 GGAGGGAAAA CTTGTCCCAC AAGAGGTTGG AATGATTTTC AGGCTAAATT  TTAGGCTTCT AAACCAAAGT CATTAAACTA GGGGAAGAGG GATAATTGTC 3500
3501 TGCCTAGGGA GGGTTTTGTG GAAGTACAGT TAAAGTAGAT CACTGTAAAC  CACATTCAGA GATGGGACCA GACTGGAAAT AAAACCTAAG AACATTTTTG 3600
3601 CTCAACTGCT TGTGAAGTTT TGGTCCCATT GTGTCCTTTG TGTGAGTTTG  TGGTGTTCAT TAGATAAATG AACTATTCCT TGTAACCCAA AACTTAAATA 3700
3701 GACGAGAACC AAAAATCTAG CTACTGTATA AGTTGAGCAA ACAGACTGAC  CTCATGTCAG ATTTGTGGGA GAAATGAGAA AGGAACAGTT TTTCTCTGAA 3800
3801 CTTGGCCTAT CTAACTGGAT CAGCCTCAGG CAGGTTTTTG TAAAGGGGGG  CACAGTGATA TGAATCACTG TGATTCACGT TCGGCTCGGG GACAAAGTTG 3900
3901 GAAATAAAAC GTAAGTAGAT TTTTGCTCAT TTACTTGTGA CGTTTTGGTT  CTGTTTGGGT AACTCGTGTG AATTTGTGAC ATTTTGGCTA AATGAGCCAT 4000
4001 TCCTGGCAAC CTGTGCATCA ATAGAAGATC CCCCAGAAAA GAGTCAGTGT  GAAAGCTGAG CGAAAAACTC GTCTTAGGCT TCTGAGACCA GTTTTGTAAG 4100
4101 GGGAATGTAG AAGAAAGAGC TGGGCTTTTC CTCTGAATTT GGCCCATCTA  GTTGGACTGG CTTCACAGGC AGGTTTTTGT AGAGAGGGGC ATGTCATAGT 4200
4201 CCTCACTGTG GCTCACGTTC GGTGCTGGGA CCAAGCTGGA GCTGAAACGT  AAGTACACTT TTCTCATCTT TTTTTATGTG TAAGACACAG GTTTTCATGT 4300
4301 TAGGAGTTAA AGTCAGTTCA GAAAATCTTG AGAAAATGGA GAGGGCTCAT  TATCAGTTGA CGTGGCATAC AGTGTCAGAT TTTCTGTTTA TCAAGCTAGT 4400
4401 GAGATTAGGG GCAAAAAGAG GCTTTAGTTG AGAGGAAAGT AATTAATACT  ATGGTCACCA TCCAAGAGAT TGGACCGGAG AATAAGCATG AGTAGTTATT 4500
```

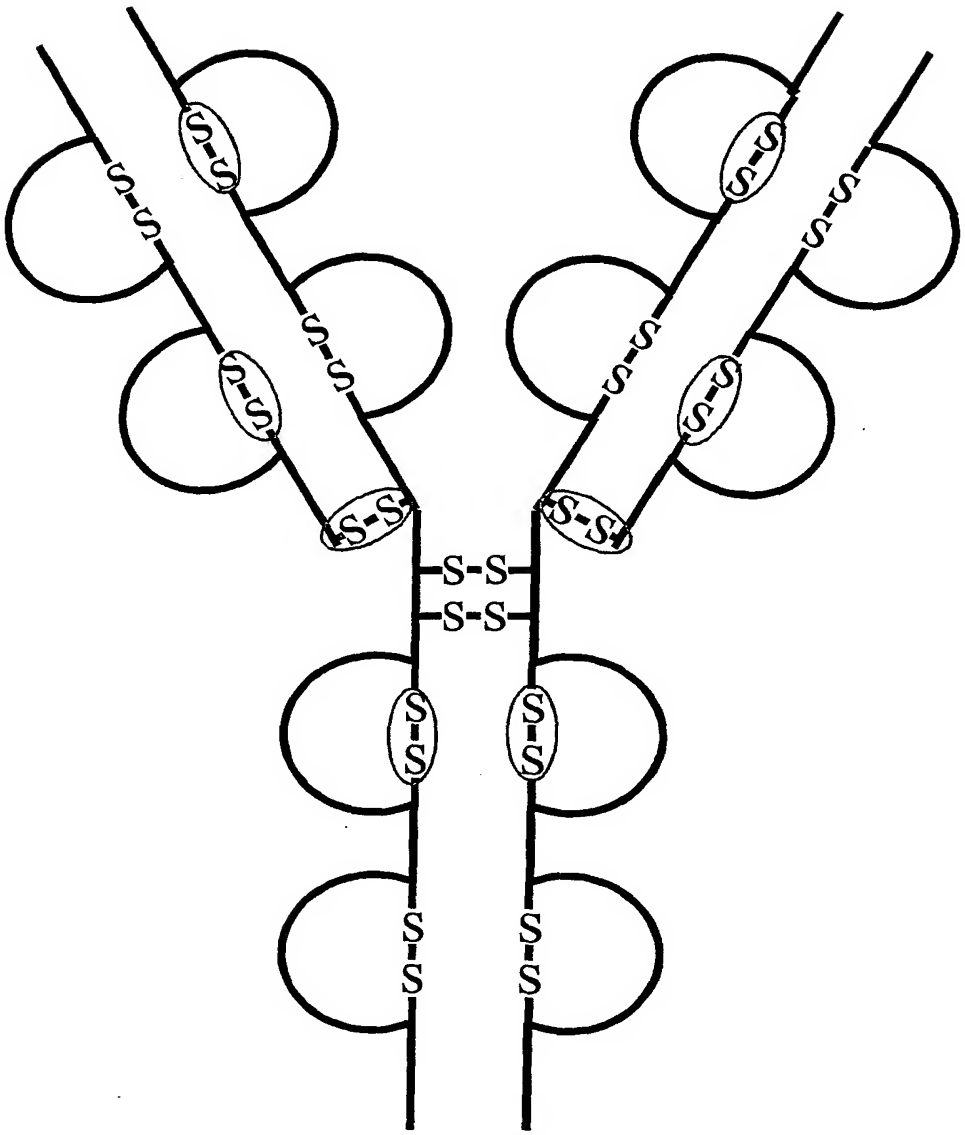


Figure 4

Schematic figure of an antibody. Disulfide bonds detected by mass spectrometry after enzymatic digest in WX-G250 are labeled by ovals.

Figure 5

Characterization of WX-G250 according to MALDI-PMF (Trypsin, LysC, AspN, GluC, and BrCN) in reflector and linear mode

Light Chain:

DIVMTQSQRF MSTTVGDRVS ITCKASQNVV SAVAWYQQKP

GQSPKLLIYS ASNRYTGVPD RFTGSGSGTD FTLTISNMQS

EDLADFFCQQ YSNYPWTFGG GTKLEIKRTV AAPSVFIFPP

SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ

ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG

LSSPVTKSFN RGEC

Heavy Chain:

DVKLVESGGG LVKLGGSLKL SCAASGFTFS NYYMSWVRQT

PEKRLELVAA INSDGGITYY LDTVKGRFTI SRDNAKNTLY

LQMSSLKSED TALFYCARHR SGYFSMDYWG QGTSVTVSSA

STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW

NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY

ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP

SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY

VDGVEVHNAK TKPREEQYNS TYRVVSVLTV LHQDWLNGKE

YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL

TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL

DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ

KSLSLSPGK

———— Reflector mode

---------- Additional information from linear mode spectra

C: Detected bridged cysteins (reflector mode)
C: Detected bridged cysteins (linear mode)
C: Cystein not determined as bridged cysteins
K: Heavy chain partially lacks C-terminal lysine

N Confirmed glycosylation site

* Confirmed deamidation sites

Light chain:

DIVMTQSQRF MSTTVGDRVS ITCKASQNVV SAVAWYQQKP GQSPKLLIYS
ASNRYTGVPD RFTGSGSGTD FTLTISNMQS EDLADFFCQQ YSNYPWTFGG
GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC

Heavy chain:

DVKLVESGGG LVKLGGSLKL SCAASGFTFS NYYMSWVRQT PEKRLELVAA
INSDGGITYY LDTVKGRFTI SRDNAKNTLY LQMSSLKSED TALFYCARHR
SGYFSMDYWG QGTSVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

Figure 6: LC-MS and LC-MS/MS of tryptic digest of cG250